bio-techne®

R&D SYSTEMS™

Quantikine® IVD® ELISA

Human Erythropoietin

Quantikine® IVD® ELISA

Human Erythropoietin Immunoassay

REF DEP00

Instructions for use in Danish, French, German, Greek, Italian, Polish, Portuguese, Spanish, and Swedish are available in a supplementary package insert located on our website at www.RnDSystems.com/go/Epo

DK Brugsanvisningerne på Dansk findes på den supplerende indlægsseddel.

FR La notice d'utilisation en Français est disponible dans le paquet d'instructions fourni en complément.

DE Die arbeitsanleitung in Deutsch finden sie in der zusätzlichen packungsbeilage.

GR Τις οδηγίες χρήσης του κιτ στα Ελληνικά θα τις βρείτε σε ξεχωριστό, επί πλέον ένθετο της συσκευασίας.

IT Le istruzioni per l'uso in Italiano sono disponibili nell'inserto aggiuntivo incluso.

PL Instrukcja obsługi w języku Polskim jęst dostępne w dodatkowym opakowaniu.

PT As instrucções de utilização em Português encontram-se nos folhetos anexos.

ES La instrucciones de utilización en Castellano están disponibles en el manual suplemetario.

SE Bruksanvisningen på Svenska finns i den kompletterande bipacksedeln.

This package insert must be read in its entirety before using this product.

CE IVD For *In Vitro* Diagnostic use.

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NAME AND INTENDED USE

Quantikine® IVD® Human Erythropoietin ELISA
R&D Systems Inc. Catalog Number DEP00

Enzyme linked immunosorbent assay (ELISA) for the quantitative determination of Erythropoietin (Epo) concentration in human serum and plasma as an aid in the diagnosis of anemia and polycythemia.

SUMMARY AND EXPLANATION

Erythropoietin, a glycoprotein (~30,400 Daltons) produced primarily by the kidney, is the principal factor regulating red blood cell production (erythropoiesis) in mammals. Renal production of Epo is regulated by changes in oxygen availability. Under conditions of hypoxia, the level of Epo in the circulation increases and this leads to increased production of red blood cells.

The over-expression of Epo may be associated with certain pathophysiological conditions (1, 2). Polycythemia exists when there is an overproduction of red blood cells (RBCs). Primary polycythemias, such as polycythemia vera, are caused by Epo-independent growth of erythrocytic progenitors from abnormal stem cells and low to normal levels of Epo are found in the serum of affected patients. On the other hand, various types of secondary polycythemias are associated with the production of higher than normal levels of Epo. The overproduction of Epo may be an adaptive response associated with conditions that produce tissue hypoxia, such as living at high altitude, chronic obstructive pulmonary disease, cyanotic heart disease, sleep apnea, high-affinity hemoglobinopathy, smoking, or localized renal hypoxia (1, 2). In other instances, excessive Epo levels are the result of production by neoplastic cells. Cases of increased Epo production and erythrocytosis have been reported for patients with renal carcinomas (3), benign renal tumors (4), Wilms' tumors, hepatomas (5), liver carcinomas (6), cerebellar hemangioblastomas (3, 7, 8), adrenal gland tumors (9), smooth muscle tumors (3, 9), and leiomyomas (10).

Deficient Epo production is found in conjunction with certain forms of anemias. These include anemia of renal failure and end-stage renal disease (1, 2, 11), anemias of chronic disorders [chronic infections (1), autoimmune diseases (1), rheumatoid arthritis (12), AIDS (13), malignancies (14)], anemia of prematurity (2), anemia of hypothyroidism (2), and anemia of malnutrition (2). Many of these conditions are associated with the generation of IL-1 and TNF-α, factors that have been shown to be inhibitors of Epo activity (1, 15). Other forms of anemias, on the other hand, are due to Epo-independent causes and affected individuals show elevated levels of Epo (2). These forms include aplastic anemias, iron deficiency anemias, thalassemias, megaloblastic anemias, pure red cell aplasias, and myelodysplastic syndromes.

Methods for determining Epo concentration in serum have been based historically on the ^{59}Fe exhypoxic polycythemic mouse *in vivo* bioassay (16). The Quantikine IVD Human Epo ELISA uses a monoclonal antibody and polyclonal antibody conjugate in a sandwich ELISA format to provide a method that is quicker, more sensitive and more specific than the bioassay. The assay is designed to measure Epo levels in serum or plasma in less than 4.5 hours, or less than 2.5 hours using the shaker protocol.

ASSAY PRINCIPLE

The Quantikine® IVD® Human Epo ELISA is based on the double-antibody sandwich method. Microplate wells, precoated with a mouse monoclonal antibody specific for Epo are incubated with specimen or standard. Erythropoietin binds to the immobilized antibody on the plate. After removing excess specimen or standard, wells are incubated with a rabbit anti-Epo polyclonal antibody conjugated to horseradish peroxidase. During the second incubation, the antibody-enzyme conjugate binds to the immobilized Epo. Excess conjugate is removed by washing. A chromogen is added to the wells and is oxidized by the enzyme reaction to form a blue colored complex. The reaction is stopped by the addition of acid, which turns the blue to yellow. The amount of color generated is directly proportional to the amount of conjugate bound to the Epo antibody complex, which, in turn, is directly proportional to the amount of Epo in the specimen or standard. The absorbance of this complex is measured and a standard curve is generated by plotting absorbance versus the concentration of the Epo standards. The Epo concentration of the unknown specimen is determined by comparing the optical density of the specimen to the standard curve. The standards used in this assay are recombinant human Epo calibrated against the Second International Reference Preparation (67/343), a urine-derived form of human erythropoietin.

MATERIALS PROVIDED

SORB	**Erythropoietin Microplate** (Part 890126) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against recombinant human Epo.
CONJ	**Erythropoietin Conjugate** (Part 890127) - 21.5 mL of a rabbit polyclonal antibody against recombinant human Epo, conjugated to horseradish peroxidase with preservatives.
CAL 0	**Erythropoietin 0.0 mIU/mL Standard** (Part 890128) - 2.1 mL of a buffered protein base with preservatives.
CAL 2.5	**Erythropoietin 2.5 mIU/mL Standard** (Part 890129) - 2.1 mL of recombinant human Epo in a buffered protein base with preservatives.
CAL 5	**Erythropoietin 5.0 mIU/mL Standard** (Part 890130) - 2.1 mL of recombinant human Epo in a buffered protein base with preservatives.
CAL 20	**Erythropoietin 20.0 mIU/mL Standard** (Part 890131) - 2.1 mL of recombinant human Epo in a buffered protein base with preservatives.
CAL 50	**Erythropoietin 50.0 mIU/mL Standard** (Part 890132) - 2.1 mL of recombinant human Epo in a buffered protein base with preservatives.
CAL 100	**Erythropoietin 100.0 mIU/mL Standard** (Part 890133) - 2.1 mL of recombinant human Epo in a buffered protein base with preservatives.
CAL 200	**Erythropoietin 200.0 mIU/mL Standard** (Part 890134) - 2.1 mL of recombinant human Epo in a buffered protein base with preservatives.
DIL AS	**Erythropoietin Assay Diluent** (Part 895057) - 11 mL of a buffered protein base with preservatives. Contains sodium azide.

DIL	SPE

Specimen Diluent (Part 895058) - 26 mL of a protein stabilized buffer with preservatives.

BUF	WASH	25X

Erythropoietin Wash Buffer Concentrate (Part 895059) - 100 mL of a 25-fold concentrate with preservative.

SUBS	A

Color Reagent A (Part 895549) - 12 mL of Color Reagent A (0.01 N buffered hydrogen peroxide).

SUBS	B

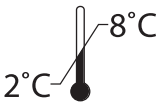
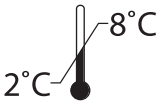
Color Reagent B (Part 895550) - 12 mL of Color Reagent B (0.35 g/L tetramethylbenzidine).

SOLN	STOP

Stop Solution (Part 895060) - 11 mL of 2 N sulfuric acid. **Caution:** *The Stop Solution with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.*

Plate Covers - 4 adhesive strips.

STORAGE

UNOPENED KIT	STORE AT 2-8° C. DO NOT USE PAST THE EXPIRATION DATE OF THE KIT.		
Opened/ Diluted Reagents	Diluted Wash Buffer	May be stored at room temperature (20-25° C) until the expiration date of the kit.	
	Stop Solution	May be stored at 2-8° C until the expiration date of the kit.	
	Specimen Diluent		
	Assay Diluent		
	Conjugate		
	Unmixed Color Reagent A		
	Unmixed Color Reagent B		
	Standards (0.0-200 mIU/mL)		
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal. May be stored at 2-8° C until the expiration date of the kit.	

WARNINGS/PRECAUTIONS

IVD For *In Vitro* Diagnostic Use

- Do not use kit reagents beyond the kit expiration date.
- For best results, each laboratory should validate a specific assay method (Benchtop or Shaker) and perform all assays by that method.
- In order to minimize within assay variation, it is recommended that the assay be pipetted within 15 minutes.
- Do not substitute kit reagents with those from other lots or other sources.
- Do not expose kit reagents to strong light during storage or incubation.
- Avoid contact of kit reagents with oxidizing agents and metal.
- Exposure to sodium azide will inactivate the conjugate.
- Do not pipette by mouth.
- Do not smoke or eat in areas where kit reagents or specimens are handled.
- Avoid contact of skin and mucous membranes with kit reagents or specimens.
- If any reagents come in contact with eyes, skin, or mucous membranes, wash with copious amounts of water and contact a physician.
- Handle all serum and materials in contact with serum in accordance with CLSI guidelines for preventing the transmission of blood-borne pathogens during laboratory procedures.
- Incubation times and temperatures other than those specified may yield erroneous results.
- Contamination of kit reagents may yield erroneous results.
- If possible, use plastic disposable pipettes, tips and containers for reagent preparation and storage. Glassware used should be rinsed thoroughly with 1 N sulfuric or 1 N hydrochloric acid followed by at least three washes of deionized water. No acid or detergent residues should remain on the glassware.
- Use polypropylene or high density polyethylene (HDPE) test tubes for specimen dilutions. **DO NOT USE GLASS TUBES.**
- Erythropoietin Assay Diluent contains sodium azide which may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal. **Note:** *Users shall report any serious incident that has occurred in relation to the device to R&D Systems®, Inc. at 1-800-343-7475 (USA and Canada) or 1-612-379-2956 and the competent authority of the Member State in which the user and/or patient is established.*

INDICATIONS OF INSTABILITY OR DETERIORATION

Color Reagents should be colorless when separate or combined. Precipitates in the reagent solutions are generally considered indications of instability or deterioration. If any of these indications of instability or deterioration are observed, or if the correlation coefficient of the standard curve is less than 0.95, retain the reagent(s) in question at 2-8 °C and contact R&D Systems, Inc. at 1-800-343-7475 (USA and Canada) or 1-612-379-2956.

OTHER SUPPLIES REQUIRED

- Pipettes and pipette tips.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm (required for the Shaker Protocol).
- 100 mL and 4 liter graduated cylinders.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent pad or paper towels for blotting the wells.
- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 600 nm.
- Immunoassay reagent trays.
- Deionized or distilled water.
- A computer capable of 4 parameter logistic curve fitting for data reduction.
- Erythropoietin Serum Control(s), *e.g.*, Quantikine® IVD® Human Serum Control 1 and 2, and Control 3 [available through R&D Systems®, Catalog # CEP01 (5 vials each of level 1 and 2) and Catalog # CEP03 (10 vials of level 3), respectively] or equivalent.

INSTRUMENTS

Assay results are measured spectrophotometrically at 450 nm using a microplate reader. For best results, a reference wavelength of 600 nm (540 nm, 570 nm, and 650 nm may also be used) should be included in the measurement to correct for optical imperfections in the polystyrene microplate. Instruments without reference filters may be used, but assay precision may decrease. A microplate reader with an optical density range of 0-3 Optical Density (O.D.) and an accuracy of ± 0.005 O.D. is recommended. Microplate readers with an O.D. range less than 0-3 O.D. may be used, but the range of the assay may be reduced.

LIMITATIONS

- The results of this assay should be used in conjunction with information available from clinical evaluations and other diagnostic procedures.
- No drugs have been investigated for assay interference.
- If specimens generate values higher than the highest standard, dilute the specimens in Specimen Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

SPECIMEN COLLECTION AND STORAGE

Serum - Use a serum separator or clot tube and allow specimens to coagulate at room temperature (20-25° C). Centrifuge at 760 x g* for 15 minutes at room temperature within 30 minutes of collection to avoid hemolysis. Aliquot and store at 2-8 °C for up to 7 days or indefinitely at ≤ -10 °C in a non-self-defrosting freezer. **Avoid repeated freeze-thaw cycles.**

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge specimens at 760 x g* for 15 minutes at room temperature within 30 minutes of collection. Aliquot and store at 2-8 °C for up to 7 days or indefinitely at ≤ -10 °C in a non-self-defrosting freezer. **Avoid repeated freeze-thaw cycles.**

It is recommended that each laboratory standardize the assay using either serum or EDTA plasma specimens.

Lipemic, grossly hemolyzed, or contaminated specimens may yield inaccurate results and should not be tested with this procedure. Further, no drugs have been investigated for assay interference.

Refer to CLSI Guideline: *Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests,* (CLSI Document GP44; 4th Edition)

* $g = (1.118 \times 10^{-5})$ (radius in cm) $(rpm)^2$

REAGENT PREPARATION

Bring all reagents to room temperature (20-25 °C) before use.

Wash Buffer (1X) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 2500 mL of Wash Buffer (1X).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well. Discard any unused, prepared Substrate Solution.

SUBSTRATE SOLUTION PREPARATION BY ASSAY SIZE		
Total Number of Wells for Assays	Volume of Color Reagent A	Volume of Color Reagent B
96	11 mL	11 mL
48	6 mL	6 mL
32	4 mL	4 mL

ASSAY PROCEDURE

Bring all reagents and specimens to room temperature (20-25 °C) before use. It is recommended that all standards, controls, and specimens be assayed in duplicate. Benchtop and shaker protocols are provided. The same protocol must be used throughout the assay.

1. Prepare all reagents as directed in the previous section.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Pipette 100 µL of Erythropoietin Assay Diluent into each well.
4. Add 100 µL of standard, control, or specimen per well. Gently tap the plate frame for approximately 1 minute to mix the well contents. Cover the plate with the adhesive strip provided. A plate layout containing a sample diagram of standards, controls, and specimens is shown on page 17.
   For Benchtop Protocol: Incubate for 2 hours ± 5 minutes at room temperature.
   For Shaker Protocol: Incubate for 1 hour ± 5 minutes at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Thoroughly aspirate or decant the contents from each well. Blot dry on clean paper toweling. **Do Not Wash.**
6. Add 200 µL of Erythropoietin Conjugate to each well. Cover the plate with a new adhesive strip.
   For Benchtop Protocol: Incubate for 2 hours ± 5 minutes at room temperature.
   For Shaker Protocol: Incubate for 1 hour ± 5 minutes at room temperature on a horizontal orbital microplate shaker.
7. Aspirate each well and wash, repeating the process three times for a total of 4 washes. Wash by filling each well with Wash Buffer (300-400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
8. Add 200 µL of Substrate Solution to each well (**Note:** *Substrate Solution* ***must*** *be used within 15 minutes of preparation*). Incubate for 20-25 minutes at room temperature **on the benchtop.**
9. Add 100 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density (O.D.) of each well within 15 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 600 nm. If wavelength correction is not available, subtract readings at 600 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

ASSAY PROCEDURE SUMMARY

❶ Prepare all reagents as instructed.

❷ Add 100 µL of Epo Assay Diluent to each well.

❸ Add 100 µL of standard, control, or specimen to each well.

For Benchtop Assay
Seal and incubate at RT for 2 hours.

For Shaker Assay
Seal and incubate at RT for 1 hour on the shaker.

❹ Thoroughly aspirate solution from wells. **DO NOT WASH.**

❺ Add 200 µL conjugate to each well.

For Benchtop Assay
Seal and incubate at RT for 2 hours.

For Shaker Assay
Seal and incubate at RT for 1 hour on the shaker.

❻ Aspirate and wash 4 times with 1X Wash Solution.

❼ Add 200 µL Substrate Solution to each well.
Incubate at RT for 20-25 minutes.

❽ Add 100 µL Stop Solution to each well.
Read at 450 nm within 15 minutes.
λ correction 600 nm.

CALCULATION OF RESULTS

Read the absorbance of each well on a microplate reader using 450 nm as the primary wavelength and 600 nm as the reference wavelength (540, 570, or 650 nm is acceptable).

Average the duplicate readings for each standard, control, and specimen and subtract the average 0 mIU/mL standard optical density.

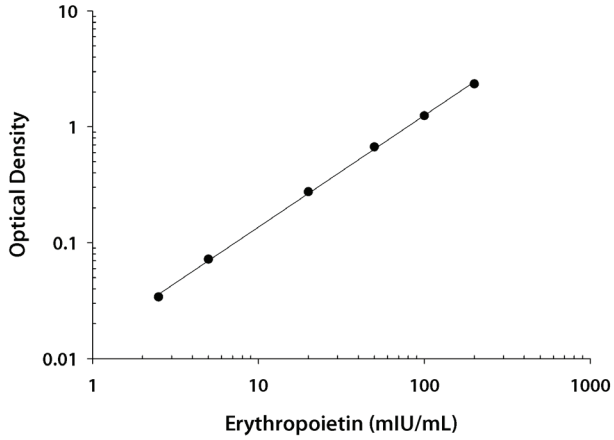
Create a standard curve by reducing the data using software capable of generating a four parameter logistic (4PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y axis against the concentration on the x axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Epo concentrations versus the log of the O.D., and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Report values for each unknown that reads within the range (2.5-200 mIU/mL) of the assay. For unknown values above the range, see Dilution of Specimens with High Epo Concentrations section. For values below the range, report as undetectable or < 2.5 mIU/mL.

TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of specimens assayed.

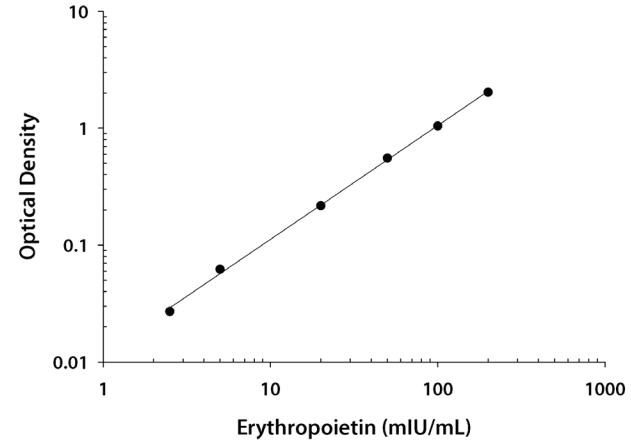
BENCHTOP PROTOCOL



BENCHTOP PROTOCOL

(mIU/mL)	O.D.	Average	Corrected
0	0.072		
0	0.074	0.073	—
2.5	0.106		
2.5	0.108	0.107	0.034
5	0.144		
5	0.146	0.145	0.072
20	0.342		
20	0.353	0.348	0.275
50	0.743		
50	0.746	0.744	0.671
100	1.298		
100	1.340	1.319	1.246
200	2.366		
200	2.463	2.414	2.341

SHAKER PROTOCOL



SHAKER PROTOCOL

(mIU/mL)	O.D.	Average	Corrected
0	0.045		
0	0.047	0.046	—
2.5	0.070		
2.5	0.076	0.073	0.027
5	0.107		
5	0.108	0.108	0.062
20	0.263		
20	0.263	0.263	0.217
50	0.597		
50	0.608	0.602	0.556
100	1.081		
100	1.098	1.090	1.044
200	2.008		
200	2.136	2.072	2.026

DILUTION OF SPECIMENS WITH HIGH EPO CONCENTRATIONS

If a serum or plasma specimen is above 200 mIU/mL, dilute it with the Specimen Diluent. For example:

- For specimens with Epo concentrations between 200 mIU/mL and 2000 mIU/mL, a 10-fold dilution of the specimen is necessary. Dilute 25 µL of specimen with 225 µL of the Specimen Diluent.
- For specimens with Epo concentrations in excess of 2000 mIU/mL, a higher dilution will be necessary to bring them within the range of the standard curve (*i.e.* 20-fold, 40-fold, *etc.* dilution).

Note: Use polypropylene or high density polyethylene (HDPE) test tubes for specimen dilutions. **DO NOT USE GLASS TUBES.** The use of glass tubes will cause erroneous results due to adsorption of Epo to the glass.

To determine the Epo concentration of the serum or plasma specimen, multiply the result obtained by the dilution factor.

QUALITY CONTROL

Each testing laboratory should establish a quality control program to monitor the performance of the Quantikine IVD Human Epo Immunoassay. As part of this program, controls with known Epo concentrations (available from R&D Systems) should be run in each assay. R&D Systems® recommends that at least two controls be run to verify the performance of the assay method. A control in the mid to upper end of the normal range and a control in the mid-region of the assay curve are good choices for the day to day evaluation of the performance of the assay. A control may also be placed at the upper end of the assay curve to evaluate the performance of the upper end of the assay. If the values obtained are not within their established ranges, the assay results may be invalid.

The results of an individual assay are valid if the control values obtained are within the published ranges of a commercially available control or the established range for an in-house control. The correlation coefficient of the fitted standard curve should be ≥ 0.95.

TROUBLESHOOTING GUIDE

Generally, assay failure is due to technical error, equipment failure or reagent failure. When an assay fails, check the expiration dates of the individual reagents and ensure that all the reagents have been stored as indicated in the product labeling. In addition, see Indications of Instability or Deterioration section for additional information. If assay performance is questionable or a problem occurs when running the assay, you may be able to isolate the problem by referring to the following table.

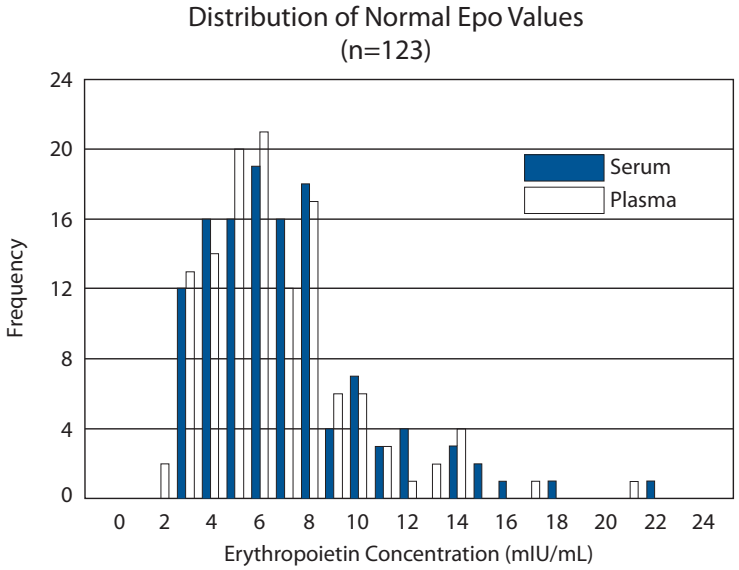
PROBLEM	POSSIBLE SOURCE	TEST OR ACTION
High % C.V.s (high variability of duplicates compared to individual laboratory precision requirements)	Incomplete washing of cells	Ensure that the wash station is working properly
	Inadequate aspiration of the wells	Wells should appear dry after aspiration
	Incomplete mixing of Color Reagent A and Color Reagent B	Ensure that Substrate Solution is adequately mixed
	Shaker splashing well contents onto plate cover	Calibrate the shaker to 500 ± 50 rpm
	Unequal volumes added to the wells	Ensure that the pipette is calibrated and working properly
Reduced low delta (< 0.015 O.D.) **or high background**	Incomplete washing of wells	Ensure that the wash station is working properly
	Inadequate aspiration of the wells	Wells should appear dry after aspiration
	Unequal volumes added to the wells	Ensure that the pipette is calibrated and working properly
	Color Reagent A and Color Reagent B mixed too early	Substrate Solution must be used within 15 minutes of preparation
Poor correlation of the Standard Curve ($r < 0.95$)	Pipetting error	Consider editing data according to individual laboratory procedures
Inadequate color development	Inadequate aspiration of the wells	Wells should appear dry after aspiration
	Unequal volumes added to the wells	Ensure that the pipette is calibrated and working properly
	Incorrect incubation times or temperatures	Adhere to recommended incubation periods and temperatures
	Conjugate or Color Reagent failure	Mix equal volumes (i.e. 100 µL each) Color Reagent A, Color Reagent B, and Epo Conjugate. Color should develop immediately
Splashing of well contents onto adhesive plate cover	Plate shaker rpm too fast	Calibrate shaker to 500 ± 50 rpm

EXPECTED VALUES

Erythropoietin concentrations were obtained from 123 normal individuals from the Minneapolis/St. Paul, Minnesota area. Using the nonparametric method for the analysis of reference values outlined in the NCCLS publication *"How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory"* (NCCLS Document C28-P; Vol. 12, No. 2) the following reference ranges (2.5-97.5 percentile) were established for Epo in serum and EDTA plasma. Each testing laboratory should establish its own normal range.

Epo Normal Ranges

Serum	EDTA plasma
3.3-16.6 mIU/mL	3.1-14.9 mIU/mL



Patients suffering from polycythemia rubra vera may have Epo concentrations within the normal range, whereas those suffering from secondary polycythemia may have elevated concentrations of serum Epo (17). Polycythemia rubra vera patients who undergo phlebotomy may have elevated serum Epo concentrations.

Patients suffering from most anemias will present with higher than normal concentrations of serum Epo, whereas those suffering from anemia associated with chronic renal failure may have serum Epo concentrations within the normal range of this assay (18). Anemic patients who receive transfusions may exhibit lower than expected serum Epo concentrations.

Abnormally high concentrations of serum Epo may also be observed in various other pathological states including renal neoplasms, benign tumors, polycystic kidney disease, renal cysts and hydronephrosis (19).

The results of this assay should be used in conjunction with information available from clinical evaluations and other diagnostic procedures.

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of the Quantikine IVD Human Epo ELISA (minimum detectable dose) is typically less than 0.6 mIU/mL. This was determined by adding two standard deviations to the mean O.D. of twenty replicates of the zero standard and calculating the corresponding concentration from the standard curve.

Specificity

The complete sequence of the Epo protein was compared with sequences in the Protein Identification Resource and the Swiss-Protein data bases. Recombinant and natural human Epo sequences are identical; no significant homology with other human proteins was found. When assayed in the Quantikine IVD Human Epo ELISA, the WHO standard 88/574 (recombinant human Epo) showed similar reactivity relative to WHO standard 67/343 (natural human Epo).

Each of the following analytes was spiked to 1 µg/mL in Specimen Diluent and run as an unknown in the assay. No cross-reactivity was observed.

Recombinant human:

ANG
β-ECGF
FGF basic
GROα
IFN-γ
IGF-I
IGF-II
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-6 R
IL-8
IL-9
IL-10
IL-11
LIF
MCP-1
M-CSF
MIP-1α
MIP-1β
OSM
PDGF-AA
PDGF-AB
PDGF-BB
RANTES
SLPI
TGF-β3
TNF-α
TNF RI

Recombinant mouse:

EGF
IL-1β
IL-3
IL-4
IL-5
IL-9
MIP-1α
MIP-1β
SCF
TNF-α

Recombinant canine:

TGF-β3

Recombinant amphibian:

TGF-β5

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine TGF-β1
porcine TGF-β1.2
porcine TGF-β2

Interference was tested by spiking serum, plasma or Specimen Diluent with selected amounts of the following substances and analyzing for the presence of Epo in the Quantikine IVD Human Epo ELISA using both the benchtop and shaker protocols. Endogenous and additional levels of these substances are detailed in the following table. To determine if the added substance interfered with assay performance, the recovery of Epo levels in each specimen was calculated. Recoveries averaged 102.5 ± 4.7% and ranged from 88.6-117.7%.

Protein	Normal Range*	Amount Added
α-1-acid glycoprotein	50-140 mg/dL	30 and 80 mg/dL
α-1-antitrypsin	150-400 mg/dL	200 mg/dL
α-2-macroglobulin	70-430 mg/dL	36 mg/dL
Albumin	3.5-5.0 g/dL	3 g/dL
Bilirubin (unconjugated)	0-0.8 mg/dL (adult)	20 mg/dL
Gamma Globulin	0.7-1.5 g/dL	3 and 5 g/dL
Hemoglobin	< 2.5 mg/dL	15, 30, and 45 mg/dL
Triglycerides	35-160 mg/dL	~2920 and ~14600 mg/dL
Transferrin	200-400 mg/dL	200 mg/dL

* Tietz, Fundamentals of Clinical Chemistry, 2nd Edition, Copyright 1976

Accuracy

1. Recovery was estimated by addition of recombinant human Epo into ten plasma and ten serum specimens. The percent recovery of the added Epo was calculated from the equation:

$$\% \text{ recovery} = \frac{\text{measured value after addition - measured value before addition}}{\text{measured value of the added material}}$$

Mean recoveries are shown in the following table. The overall mean for the two specimen types and two assay protocols was 100 ± 12%

Specimen Type	Protocol	n	Amount Added	Mean Recovery
Serum	Benchtop	10	52.2 mIU/mL	100%
	Shaker	10	57.5 mIU/mL	93%
Plasma (EDTA)	Benchtop	10	51.8 mIU/mL	102%
	Shaker	10	55.9 mIU/mL	105%

2. To determine whether the Quantikine IVD Human Epo ELISA exhibits a high dose hook, a specimen containing Epo at a concentration twenty-fold higher than the highest kit standard was serially diluted and measured in triplicate using both the benchtop and shaker assay protocols. No high dose hook was observed at 4000 mIU/mL for either protocol.

3. Five matched serum and plasma specimens containing elevated Epo concentrations were diluted with Specimen Diluent and assayed using the Quantikine IVD Human Epo ELISA. Diluted specimens demonstrated very good linearity when compared to neat concentrations of Epo.

		Benchtop Protocol		Shaker Protocol	
		EDTA Plasma	Serum	EDTA Plasma	Serum
1:2	Average % of Expected	102	101	101	103
	Range (%)	95-115	95-109	96-110	98-110
1:4	Average % of Expected	103	104	101	102
	Range (%)	97-123	100-117	92-111	92-111
1:8	Average % of Expected	105	104	102	101
	Range (%)	96-132	94-126	91-114	91-109
1:16	Average % of Expected	103	99	97	95
	Range (%)	93-129	87-126	88-106	81-110

Human Serum versus EDTA Plasma

Matched EDTA plasma and serum specimens from 153 subjects were assayed using the Quantikine® IVD® Human Epo ELISA. Results from the assay demonstrated a high correlation (r=0.9928) between the EDTA plasma and serum specimens.

Assay Precision

Assay precision was assessed as outlined in the NCCLS publication *"Evaluation of Precision of Clinical Chemistry Devices - Second Edition"* (NCCLS Document EP5-T2; Vol. 12, No. 4). Two modifications were made to the basic design described in the NCCLS guideline. The number of assays performed was changed from two assays per day for twenty days to one assay per day for thirty days. Additionally, three reagent lots were used instead of one. Four control pools were run in both the benchtop and shaker protocols of the Quantikine IVD Human Epo ELISA. Thirty assays were run using each protocol over a thirty day period with three separately manufactured lots of reagents. The results of each assay were tabulated and the within assay precision and total assay precision of each control pool was calculated.

	Benchtop Protocol (n=30)				Shaker Protocol (n=30)			
Control	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Mean (mIU/mL)	5.82	16.01	25.18	79.02	6.45	16.22	26.35	84.14
Within Assay Precision (%CV)	4.95	2.84	5.22	3.06	7.82	3.02	2.18	2.42
Total Precision (%CV)	8.25	4.30	5.90	4.24	10.30	3.74	4.20	3.71

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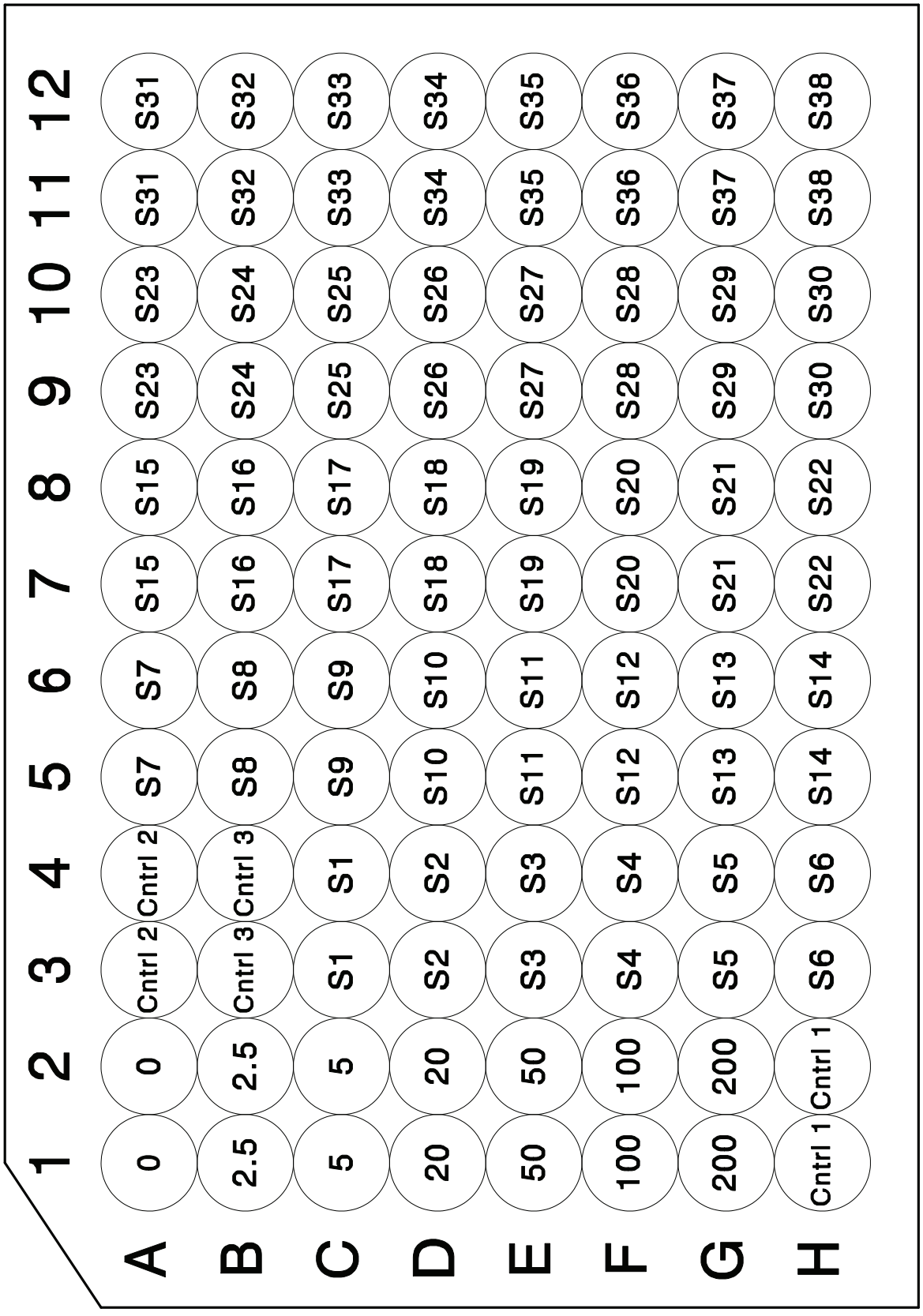
IVD CE

PLATE LAYOUT

A sample diagram for standards, controls, and specimens is shown below.



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